UVB Radiation and Human Monocyte Accessory Function: Differential Effects on Pre-Mitotic Events in T-Cell Activation

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Purified T lymphocytes fail to proliferate in response to antigenic and mitogenic stimuli when cultured in the presence of accessory cells that have been exposed in vitro to sublethal doses of UVB radiation. Because proliferation represents a final stage in the T-cell activation process, the present study was conducted to determine whether T cells were able to progress through any of the pre-mitotic stages when UVB-irradiated monocytes were used as model accessory cells. In these experiments, monoclonal anti-CD3 antibodies were employed as the mitogenic stimulus. Culture of T cells with UVB-irradiated monocytes did allow the T cells to undergo an increase in intracellular free calcium, which is one of the first steps in the activation sequence. The T cells expressed interleukin-2 receptors, although at a reduced level. However, T cells failed to produce interleukin-2 above background levels when they were placed in culture with monocytes exposed to UVB doses as low as 50 J/m^2. Incubation of T cells with UVB-irradiated monocytes did not affect the subsequent capacity of T cells to proliferate, since they developed a normal proliferative response in secondary culture when restimulated with anti-CD3 antibodies and unirradiated monocytes. These studies indicate that T lymphocytes become partially activated when cultured with UVB-irradiated monocytes and mitogenic anti-CD3 monoclonal antibodies. In addition, they suggest that interleukin-2 production is the T-cell activation step most sensitive to inhibition when UVB-irradiated monocytes are employed as accessory cells. *J Invest Dermatol 94:204–209, 1990*

In recent years, there has been increasing awareness that exposure to environmental agents may have unsuspected effects on the human body. Ultraviolet radiation has been shown to exhibit profound influences on selected cell-mediated immune responses, which, in turn, are thought to facilitate the appearance of ultraviolet radiation-induced skin cancers (reviewed in Refs [1] and [2]). Although UV-induced changes in the immunologic function of natural killer cells [3,4], keratinocytes [5], and the circulating patterns of lymphocytes [6,7] may also play a role in the immunopathogenesis of ultraviolet-radiation carcinogenesis, it is the function of antigen presenting, or accessory, cells for both helper and suppressor T cells that has received the most scrutiny [8–13]. These cells, which are present in the skin as epidermal Langerhans cells and dermal macrophages, are essential for the activation and expansion of those clones of helper and cytotoxic T lymphocytes that ultimately mediate anti-tumor immune responses.

T-cell activation is a complex process which requires both the participation of an initiating stimulus (antigen or mitogen) and an antigen-presenting, or accessory, cell [14,15]. The precise role which accessory cells play in T-lymphocyte activation is only partially understood. At least three essential macrophage-derived activities have been identified [16,17]. These include: 1) the processing of antigen, a step in which antigen is denatured in acidic vesicles and re-expressed on the cell surface; 2) the production of various cytokines, including interleukin-1 alpha (IL-1 alpha), interleukin-1 beta (IL-1 beta), and interleukin-6 (Krutmann J, Kirnbauer R, Schwarz T, May LT, Seghal PR, Luger TA: Interleukin 6: a novel accessory cell signal involved in human T-lymphocyte activation [submitted]), that act as necessary co-factors in the antigen presentation process; and 3) surface expression of class II major histocompatibility determinants that serve as binding sites for some processed antigens and are recognition sites for T cells engaged in class II restricted functions.

Antibodies to the CD3 antigen act as mitogenic stimuli for T lymphocytes [18], and have been particularly useful in dissecting

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Abbreviations:

$[Ca^{2+}]_i$: cytoplasmic free calcium
EDCI: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
FCS: fetal calf serum
Fura-2AM: Fura-2-tetrakis (aretoxymethyl) ester
HBSS: Hank's balanced salt solution
IL-1 alpha: interleukin-alpha
IL-1 beta: interleukin-1 beta
IL-2: interleukin-2
IL-6: interleukin-6
MN: monocytes
MN(UV): ultraviolet-irradiated monocytes
MoAb: monoclonal antibody
PBMC: peripheral blood mononuclear cells
PHS: pooled human serum
UVB: ultraviolet B

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out the various changes in T cells which eventuate in their fully activated state [15]. Stimulation of T lymphocytes by anti-CD3 antibodies causes a rapid increase in the intracellular concentration of cytoplasmic free calcium ($[Ca^{2+}]_i$. This occurs rapidly and appears to be due to the generation of inositol triphosphate [19–21]. When phytohemagglutinin is used as a mitogenic stimulus, these initial events in T-cell activation are followed by the expression of receptors for interleukin-2 on the cell surface membrane [22]. The same cells subsequently produce and secrete the lymphokine interleukin-2 (IL-2). IL-2 binds in an autocrine fashion to IL-2 receptors [17]. As a result, T-cell proliferation occurs and is a final stage in the activation process [23–25].

Many of the events in anti-CD3-induced T-cell activation depend on the presence of accessory cells [26]. Moreover, it appears that the various pre-mitotic events in T-cell activation have different requirements for the various accessory signals [18,23]. Using human peripheral blood monocytes as a model for UVB-induced defects in accessory function, we have reported that in vitro exposure of human peripheral blood monocytes to UVB radiation (290–320 nm) renders them incapable of acting as accessory cells for anti-CD3-induced T-cell activation [27]. These studies assessed T-cell proliferation by measuring tritiated thymidine incorporation as an index of DNA synthesis. However, as was mentioned, this represents a relatively late stage in the T-cell activation cascade. The present study was designed to examine the effects of UVB irradiation of accessory cells on earlier, premitotic events in anti-CD3-induced T-cell activation, and to determine, when UV-irradiated monocytes were used as accessory cells, the most sensitive stage in the T-cell activation process. Specifically, we examined whether T cells, cultured in the presence of UV-irradiated monocytes and anti-CD3 antibodies, were able to generate an increase in intracellular $[Ca^{2+}]_i$, to produce IL-2, and to express IL-2 receptors. We found that in vitro exposure of monocytes to UVB radiation modulated these pre-mitotic events differentially and that IL-2 production was the most sensitive step in this process.

MATERIALS AND METHODS

Antibodies and Cytokines Purified OKIa MoAb, which is directed against a framework HLA-DR determinant, and purified OKT3 MoAb, which is a mouse monoclonal antibody directed against the CD3 antigen on human T cells, were purchased from Ortho Diagnostic Systems, Inc. (Raritan, NJ). The mouse monoclonal IgG2a MoAb 64.1, which also identifies the human CD3 antigen, was generously provided by Dr. Paul Gladstone, Genetic Systems Corporation, Seattle, WA. FITC-conjugated, purified anti-Tac mAb, which identifies the IL-2 receptor (CD25) antigen on activated T cells [29], was purchased from Becton Dickinson (Mountain View, CA). Human recombinant IL-2 was obtained from AMGEN Biologicals (Thousand Oaks, CA). The biologic activity of this reagent was verified prior to its use by determining its ability to cause proliferation of the IL-2-dependent murine CTLL 20 line.

Purification of T Lymphocytes PBMC were obtained from the heparinized blood of healthy human volunteers by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density sedimentation. In most experiments, T lymphocytes were depleted of accessory cells by a four-step purification procedure. In some experiments, as indicated, only a three-step purification procedure was employed. Briefly, PBMC were allowed to adhere to 100 × 20 mm tissue-culture dishes (Falcon, Oxnard, CA) for 1 h. Plastic non-adherent cells were then placed on nylon-wool columns (Fenwall Laboratories, Deerfield, IL) for 45 min. Nylon wool non-adherent cells were subsequently exposed to the lysosomotropic agent L-leucine-methyl-ester (Sigma Chemical Co., St. Louis, MO) for 40 minutes according to the method of Thiele et al [28]. The fourth purification step consisted of treatment of T cells with a 1:50 dilution of OKIa and low toxicity rabbit complement (Cedarlane Laboratories Limited, Hornby, Ontario). Treatment in this manner resulted in a T-lymphocyte population that, without the addition of exogenous accessory cells, was completely unresponsive to Concanavalin A or anti-CD3 antibodies. The number of Ia-positive cells was less than 0.5% as monitored on a FACS EPICS V fluorescence activated cell sorter (Coulter EPICS Division, Hialeah, FL), which is at the limit of sensitivity of the instrument.

Preparation of Accessory Cells Accessory cells were prepared by gently scraping off and collecting the plastic-adherent cells during the first step of the T-lymphocyte purification procedure. They were then sedimented, resuspended in HBSS (KC Biological, Lenexa, KS) and counted. This population was 85%–90% non-specific esterase positive, and for the purpose of this paper will be called monocytes (MN).

UVB-Irradiation of Monocytes Two × 10^6 MN in 1.5 ml HBSS without phenol red were placed in 35 × 10 mm culture dishes (Falcon, Oxnard, CA) and exposed to various doses of UVB from 4 FS20 sunlamp bulbs (Westinghouse Electrical Corp., Bloomfield, NJ). The UVB output was monitored by means of an IL 700 Research Radiometer and SEE 240 UVB photodetector (International Light, Newburyport, MA) and was approximately 12.2×10^{-5} W/cm² at a tube-to-target distance of 22 cm. This detection unit is solar blind. In order to obtain an evenly irradiated cell population, culture dishes were gently shaken every 15 seconds during UVB exposure. MN were recovered from the dishes by scraping with a rubber policeman, sedimented, resuspended in RPMI-1640 medium (Whittaker, Walkersville, MD), and counted. UV-irradiated MN [MN(UV)] and non-UV-irradiated MN were treated in an identical fashion. Viability of irradiated MN, as assessed by trypan blue exclusion, was greater than 90%, and did not differ from that of unirradiated cells during the 72-h culture period.

Measurement of $[Ca^{++}]_i$ Cytoplasmic free calcium concentration was measured with the dye Fura-2 as described by Tsien et al [30]. Highly purified T lymphocytes were loaded with 4 μM of Fura-2/AM in 10 mM Hepes buffered Krebs solution for 20 min at 37°C. After loading, T cells were collected by centrifugation and resuspended to approximately 5 × 10^6 cells/ml and centrifugation and resuspended to approximately 5 × 10^6 cells/ml and incubated for at least an additional 15 min at 37°C to ensure complete de-esterification. To initiate each experiment, 5 × 10^6 cells were centrifuged for 5 seconds at 14,000 g in a microcentrifuge and resuspended in a cuvette in 1 ml of buffer solution with or without MN that had been exposed to 100 J/m² UVB. Fluorescence was measured at 37°C in an Aminco-Bowman spectrophotofluorometer (excitation, 340 nm; emission, 500 nm) equipped with a magnetic cuvette stirrer. Once a stable baseline signal was obtained, 20 μg of the anti-CD3 mAb was added as a bolus injection to the cuvette. Cytosolic free calcium was calculated before and at various time points after addition of the anti-CD3 MoAb from the Fura-2 fluorescence (F) according to Tsien et al [30], using the equation $[Ca^{++}]_i = K_d(F - F_{min})/(F_{max} - F)$, where K_d is the Fura-2 dissociation constant for Ca^{++} (224 nM) and F_{max} is the maximum fluorescence measured after addition of 40 μM digitonin, in the presence of saturating calcium (>1 nM). F and F_{max} were corrected for the presence of extracellular Fura-2 determined by measuring the fluorescence decrease on addition of 1 mM $MnCl_2$ prior to the addition of digtonin [31]. Determination of F_{max} was achieved after the addition of 40 μM digitonin by chelation of the Mn^{++} with 20 mM EDTA followed by 30 mM $CaCl_2$. An F_{min} for intracellular Fura-2 was calculated from the relationship between F_{max} and F_{min} of Fura-2 in a cell-free system and the corrected F_{max} [31]. The relationship is: F_{min}, corrected = $[(F_{max}$ corrected − AF)/B] + AF, where $B = F_{max}/F_{min}$ for Fura-2 in a cell-free system and AF = cell autofluorescence, determined as the fluorescence remaining after addition of 1 mM $MnCl_2$ and 40 μM digitonin.

Immunofluorescent Staining for IL-2 Receptor Expression One million T lymphocytes were co-cultured with 2 × 10^5 autologous MN and OKT3 in 17 × 100 mm polystyrene tubes (Fisher, Pittsburgh, PA) in a total volume of 1 ml at 37°C in 5% CO_2 for 18 h. In preliminary experiments, these culture conditions were

found to be optimal for IL-2-receptor expression. Culture medium was RPMI-1640 supplemented with 10% PHS. IL-2 receptor expression was determined using the FITC-conjugated anti-Tac mAb. Reactivity of T cells with the anti-CD25 MoAb was assessed by cytofluorometry, using a FACS EPICS V fluorescence-activated cell sorter (Coulter EPICS Division, Hialeah, FL). Data are given as % Tac positive cells, which was calculated by subtracting background fluorescence in cultures with T-cells without MN from those obtained with T cells plus OKT3 and MN. T cells treated in this manner contain less than 1%–2% Tac positive cells. Tac antigen expression in cultures with T cells alone was identical to T cells in cultures with T cells plus OKT3.

Anti-CD3-Induced IL-2 Production Two million three-step purified T lymphocytes were co-cultured with 4×105 MN and the anti-CD3 MoAb OKT3 in a total volume of 1 ml in 17×100 ml polystyrene tubes (Fisher, Pittsburgh, PA) for 24 h. The concentration of anti-CD3 antibodies and MN as well as the time point for harvesting the supernatants in order to achieve maximal IL-2 activity was determined in preliminary experiments. Culture medium consisted of RPMI-1640 supplemented with 5% FCS. The supernatants were recovered by centrifugation and stored at −20°C until assay.

Assay of IL-2 Activity IL-2 activity was determined as [^{3}H]-thymidine uptake by the IL-2-dependent murine cell line CTLL 20. CTLL 20 cells were washed extensively and were resuspended at 1×10^5 cells/ml in culture medium. One hundred microliters of sample supernatant was added to 100 μl of the cell suspension in each well of 96-well flat-bottomed microtiter plates (Falcon, Oxnard, CA). IL-2 determination was performed in triplicate. After 19 h of culture, 1 μCi of [^{3}H] thymidine was added to each well and the cells were allowed to incubate for an additional 5 h. Tritiated thymidine content was assessed as described above. IL-2 activity was calculated by modified probit analysis [32] with the IL-2 standard given a value of 1000 U/ml.

Anti-CD3-Induced T-cell Proliferation Monoclonal anti-CD3 antibodies served as the mitogenic stimulus. One hundred thousand highly purified (four-step) T cells were co-cultured with 2×10^4 autologous UV-irradiated MN and 2.5-ng OKT3 in a total volume of 200 μl of culture medium. OKT3 and MN were used in concentrations which, in preliminary experiments, gave optimal proliferative responses. Culture medium consisted of RPMI-1640, supplemented with 10% pooled human serum (PHS), penicillin (10,000 U/ml), streptomycin (10,000 ug/ml), L-Glutamine (2 mM), and Hepes Buffer (10 mM). Cultures were set up in 96-well round bottom microtiter plates (Corning, Corning, NY) and incubated for 72 h at 37°C in 5% CO_2 humidified air. All variables were performed in triplicate. One μCi of methyl-^{3}H-thymidine (New England Nuclear, Boston, MA) was added to each well for the last 18 h of culture. Wells were harvested onto glass filter paper using a Multiple Automatic Sample Harvester (M.A. Bioproducts, Walkersville, MD) and tritiated thymidine content per well was assessed by scintillation spectrophotometry. Data were expressed as cpm $\times 10^{-3} \pm$ SD.

RESULTS

Wavelength Dependence for Inhibition of MN Accessory Function by UV Radiation In previous studies we had shown that in vitro exposure of MN to sublethal doses of UVB radiation decreased their ability to function as accessory cells for anti-CD3-induced T-cell mitogenesis [27]. Proliferation was completely inhibited by irradiating MN with a dose of 100 J/m² UVB. Since FS20 lamps emit approximately 60% UVB and 40% UVA radiation, initial studies were performed to determine whether the wavelengths within the UVB were necessary for inhibition of accessory function. To address this issue, a plate-glass filter (4 mm thick) was interposed between the light source and the cells being irradiated. The plate glass filter reduced the amount of UVB radiation by greater than 99%. Because the plate glass also filtered out radiation within the UVA to some extent, irradiation times were prolonged to expose cells to the same dose of UVA as was administered without the filter. As shown in Table I, insertion of the plate-glass filter abrogated the inhibitory activity of the FS20 lamps on accessory function for anti-CD3-induced T-cell mitogenesis. These results indicated that wavelength within the UVB were required for this effect.

Table I. Wavelength Dependence of the UV-Induced Inhibition of Accessory Function for T-cell Mitogenesis by Anti-CD3 Antibodies

Culture Conditions[a]	Tritiated Thymidine Incorporation (cpm ± SD)
T cells	513 ± 370
T cells + anti-CD3	364 ± 311
T cells + MN	890 ± 261
T cells + MN + anti-CD3	20,896 ± 2075
T cells + MN(UV)	277 ± 156
T cells + MN(UV) + anti-CD3	719 ± 253
T cells + MN(filtered UV)	598 ± 126
T cells + MN(filtered UV) + anti-CD3	25,975 ± 2320

[a] One hundred thousand T cells were placed in primary culture with anti-CD3 and 2×10^4 monocytes that: 1) were not UV-irradiated; 2) were UV-irradiated with unfiltered FS20 lamps [MN(UV)]; or 3) were irradiated with window-glass-filtered UV from FS20 lamps [MN(filtered UV)]. Cells were cultured for 72 h and pulsed with tritiated thymidine over the final 18 h. T-cell proliferation was assessed as described in *Materials and Methods*.

Effect of In Vitro Ultraviolet B Irradiation of MN Accessory Cells on Anti-CD3 Antibody-Induced $[Ca^{++}]_i$ in T cells One of the earliest steps in the T-lymphocyte activation process is an increase in intracellular concentration of $[Ca^{++}]$ [19,21]. This step in T-cell activation occurs within seconds to minutes after binding of anti-CD3 MoAb to the CD3 molecules on T cells and does not appear to be accessory dependent. Therefore, this experiment also assessed whether UVB-irradiated MN might have a suppressive effect, independent of accessory function, on this initial step in T-cell activation. Alterations of $[Ca^{++}]_i$ in T cells were monitored using the fluorescent Ca^{++} indicator fura-2 [33]. Highly purified T lymphocytes were loaded with fura-2 and subsequently stimulated with anti-CD3 MoAb in the presence of irradiated or unirradiated MN. Addition of anti-CD3 antibodies to T cells and unirradiated MN led to an increase in $[Ca^{++}]_i$ which could be observed within minutes and which reached a plateau within 6 min following addition of anti-CD3 antibodies (Table II). Cells were monitored for a total time of 25 min, but no further increase in $[Ca^{++}]_i$ could be detected (data not shown). Stimulation of T cells with anti-CD3 in the presence of MN which had been exposed to 100 J/m² UVB gave

Table II. Anti-CD3 Antibody-Induced Rise in Cytoplasmic Free Calcium Concentration in T Cells Cultured with UVB-Irradiated and Unirradiated Monocytes[a]

		$[Ca^{++}]_i$ (nM)		
			Peak Response	
Experiment	T Cell Culture	Pre-Anti-CD3 Antibody	Post-Addition of Anti-CD3 Antibody	Percent Increase
1	MN (no UV)	95	112	18
	MN (UV)	127	158	24
	buffer	102	121	19
2	MN (no UV)	104	126	21
	MN (UV)	77	90	17

[a] Fura-2 loaded, purified T cells were incubated with unirradiated MN, UVB-irradiated (100 J/m²) MN, or with no MN for up to 10 min. Once a baseline fluorescent signal (pre-anti-CD3 antibodies) was obtained, 20 μg of MoAb was added. Data are given as $[Ca^{++}]_i$ in nM, calculated from the fluorescence signal obtained before and after the addition of anti-CD3 antibody as described in detail in *Materials and Methods*.

a similar response. Furthermore, anti-CD3 increased $[Ca^{++}]_i$ in an almost identical fashion when added to T cells in the absence of any MN. Thus, UVB-irradiation of human MN accessory cells did not affect the anti-CD3-induced alterations of $[Ca^{++}]_i$ in T cells, an activation step which is independent of signals provided by accessory cells. To demonstrate the specificity of the increase for these antibodies, a control monoclonal antibody was added that was not expected to activate the T cell at time zero. As expected, no increase in $[Ca^{++}]_i$ was observed (data not shown).

Effects of In Vitro UVB Irradiation of MN Accessory Cells on IL-2 Receptor Expression by Anti-CD3 Stimulated T cells We next assessed the effect of MN (UV) on IL-2 receptor expression on T cells, since this represents a later step in the T-cell activation cascade than the increase in $[Ca^{++}]_i$. This was examined by determining the reactivity of T cells with anti-Tac, a monoclonal antibody that specifically recognizes the CD25 antigen of the human IL-2 receptor [34]. In cultures with unirradiated MN and anti-CD3 antibodies, maximal IL-2 receptor expression could be detected after 18 h of culture. Table III and Fig 1 show that when MN were exposed in vitro to various doses of UVB, a decrease in the number of IL-2-receptor positive T cells could be observed. However, even doses up to 200 J/m² UVB failed to reduce IL-2 receptor expression to background levels. Human peripheral blood monocytes, when stimulated with gamma-interferon, have been shown to express receptors that are capable of binding IL-2 [34,35]. To exclude the possibility that UVB radiation induced IL-2 receptors on MN, MN(UV) were stained for IL-2 receptors. No IL-2 receptors could be detected on MN following exposure to UVB irradiation (data not shown), indicating that Tac antigen staining in these cultures was solely caused by T-lymphocyte expression of that antigen.

Effects of In Vitro UVB Irradiation of MN Accessory Cells on IL-2 Production by Anti-CD3-Stimulated T-Cells The effect of MN(UV) on IL-2 production by anti-CD3-stimulated T lymphocytes was next examined. Table IV shows that there was a dose-dependent inhibition of IL-2 elaboration by T cells when MN were exposed to incremental doses of UVB. 50 J/m² UVB was sufficient to reduce IL-2 activity in culture supernatants to background levels. Thus, IL-2 elaboration by T cells was found to be exquisitely sensitive to UVB doses delivered to the accessory cell, and required smaller UVB doses (50 J/m²) than those employed to completely inhibit T-cell blastogenesis by anti-CD3 MoAb or to inhibit IL-2 receptor expression on T cells.

Lack of an Effect of In Vitro UVB Irradiation of MN Accessory Cells on Subsequent Immunologic Responsiveness of T Cells Experiments were next conducted to determine whether pre-incubation of T cells with UVB-irradiated monocytes and anti-CD3 antibodies had an effect on the proliferative capacity of responding T cells in secondary culture. T lymphocytes were incubated in primary culture with UV-irradiated monocytes and anti-CD3 antibodies. Twenty-four hours later, the cells from the primary culture were harvested and were then placed in secondary culture for 72 h with fresh autologous monocytes and anti-CD3 antibodies. As shown in Table V, the proliferative response of T

Table III. Tac Antigen Expression on T Cells in Response to Anti-CD3 Antibodies When Cultured with UVB-irradiated Monocytes

UVB Treatment of Monocytes[a]	Percent Tac Positive T Lymphocytes (Mean + SEM)
0 J/m² (n = 4)	32.0 ± 5.3
50 J/m² (n = 2)	17.7 ± 5.2
100 J/m² (n = 3)	13.9 ± 3.0
200 J/m² (n = 3)	12.7 ± 3.3

[a] All cultures also contained T lymphocytes and anti-CD3 antibodies as described in *Materials and Methods*. Number of experiments is given in parentheses.

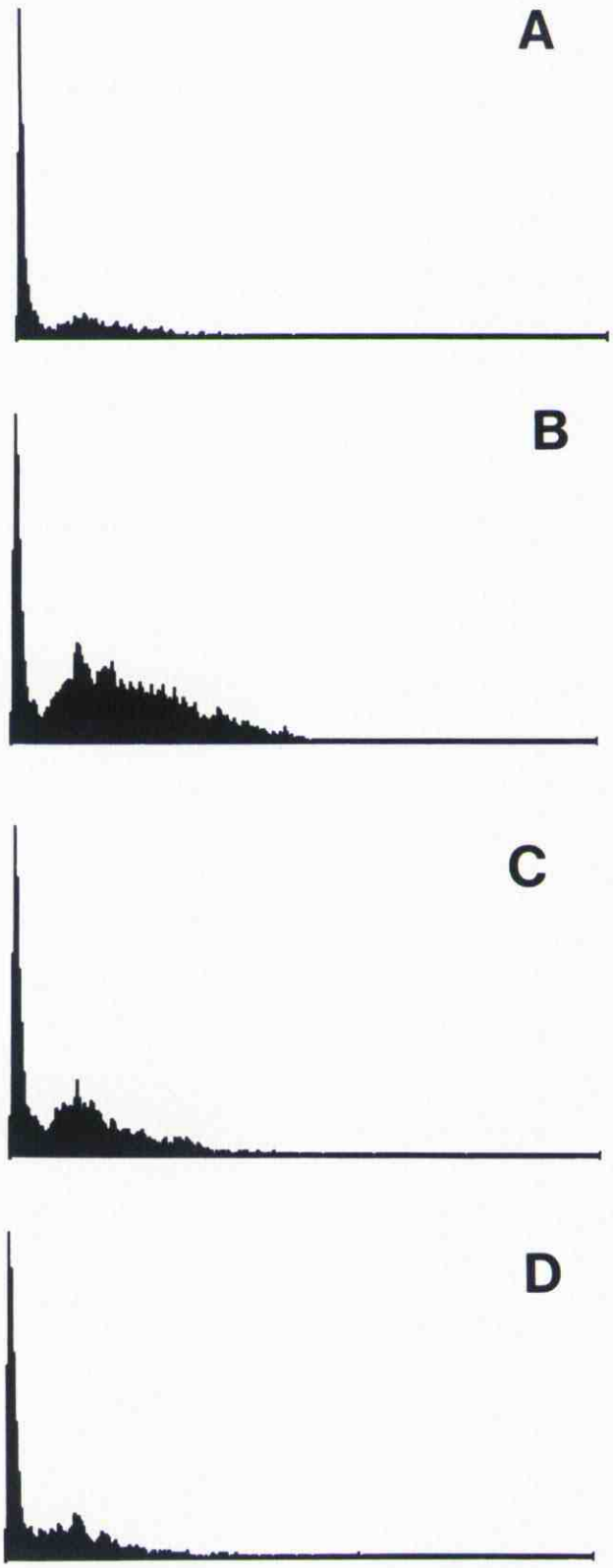

Figure 1. Histograms of T lymphocytes stained with anti-Tac monoclonal antibodies in cultures of (*A*) T cells with OKT3; (*B*) T cells with OKT3 and unirradiated monocytes; (*C*) T cells with OKT3 and monocytes exposed to 100 J/m² UVB; and (*D*) T cells with OKT3 and monocytes exposed to 200 J/m² UVB. Fluorescence intensity is located along the abscissa and cell number is located along the ordinate.

Table IV. Effect of UVB-Irradiation of Monocytes on T-Cell Production of IL-2 in Response to Anti-CD3 Monoclonal Antibodies[a]

UVB Treatment of Monocytes[b]	Il-2 Activity (U/ml)[c]	p Value[d]
0 J/m² (n = 5)	20.42 ± 7.1	
25 J/m² (n = 5)	8.26 ± 3.7	<0.01
50 J/m² (n = 5)	0.48 ± 0.21	<0.005
100 J/m² (n = 5)	0.18 ± 0.08	<0.005
200 J/m² (n = 4)	0.63 ± 0.63	<0.005

[a] Cultures were prepared as described in *Materials and Methods* using monocytes, exposed to various doses of UVB radiation, as stimulator cells. Supernatants from the various cultures were removed and incubated with CTLL 20 cells for 24 h. The cells were pulsed with tritated thymidine over the final 5 h of culture. Units of IL-2 activity were calculated by modified probit analysis.

[b] Number of experiments at each UVB dose is given in parentheses.

[c] Mean + SD.

[d] Compared to 0 J/m² UVB.

cells that had been placed in primary culture with UVB-irradiated monocytes was the same as that of monocytes placed in primary culture with unirradiated monocytes. We thus concluded that prior incubation with UVB-irradiated accessory cells had no effect on the capacity of T cells to respond to subsequent immunologic stimuli.

DISCUSSION

These studies have demonstrated that T lymphocytes become partially activated when UVB-irradiated peripheral blood monocytes are employed as accessory cells for anti-CD3 antibody-induced T-cell activation. This was most apparent when IL-2 production and IL-2 receptor expression by responding T lymphocytes were compared. T-lymphocyte production of IL-2 could not be detected in culture supernatants when monocytes, exposed to as small a dose of UVB as 50 J/m², were employed as accessory cells. In contrast, there was partial IL-2 receptor expression by T cells when stimulator monocytes were exposed to four times that dose. IL-2 receptor expression can first be detected in the G1a phase of the cell cycle, whereas IL-2 production occurs during the G1b portion of the cell cycle [36]. IL-2 production provides the stimulus for cells to enter and move through G1b and enter the S phase. The demonstration that IL-2 receptors were present on a certain percentage of T lymphocytes when co-cultured with UVB-irradiated accessory cells, but that IL-2 secretion was not detected, would imply a maturation arrest of T cells that occurred in the G1a stage of the cell cycle.

Exactly why UVB-irradiated accessory cells were able to provide some signals for T-lymphocyte activation but not others is unknown, but may relate to differences in the accessory requirements for IL-2 production and IL-2 receptor expression. It is possible that UVB-irradiated monocytes were unable to provide an immobilized matrix necessary for full T-cell activation. A number of studies have demonstrated that anti-CD3 antibodies require such a substrate for full T-cell activation [23,37]. Using human T-cell clones, Meuer et al demonstrated that soluble anti-CD3 antibodies were able to induce IL-2 receptor expression but were an insufficient stimulus for IL-2 production [23]. T-cell proliferation could be achieved when the anti-CD3 antibodies were attached to a solid matrix such as sepharose beads. Similarly, Manger et al found that the Jurkat cell line, which phenotypically resembles human resting T cells, could be activated by immobilized anti-CD3 antibodies, but not by soluble anti-CD3 antibodies [37]. In our system, immobilization of anti-CD3 antibodies could be attributed at least in part to antibody binding to monocyte Fc receptors. If this is the case, then it might be expected that UVB-exposure would inhibit the ability of monocytes to bind to anti-CD3 antibodies attached to T cells. In other studies, we have demonstrated that UVB inhibits the binding of T cells to monocytes, a step necessary for T-cell proliferation to occur (Krutmann et al, submitted).

Jenkins and Schwartz [38] have recently demonstrated that preincubation of murine T cells with antigen and splenic adherent antigen presenting cells that have been treated with the chemical cross-linking agent 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDCI) results in antigen-specific T-cell tolerance. When T cells treated in this manner are harvested from primary culture and re-cultured with antigen and non-EDCI-treated macrophages, they fail to develop a normal proliferative response to the tolerizing antigen, although proliferation can be achieved if other antigens are substituted in the primary culture. Similar results have been shown to occur when class II bearing keratinocytes are employed as the antigen-presenting cell [39]. In the case of ECDI-treated antigen-presenting cells, it has furthermore been shown that an increase in levels of intracellular free calcium are essential for production of unresponsiveness [40]. Although intracellular free calcium in T cells was found to rise both in our studies and those of Jenkins [40], it appears that the defect in accessory cells imposed by these two different types of treatment (i.e., UVB radiation and EDCI cross linking) are distinct, since T cells incubated in primary culture with UVB-treated accessory cells are fully capable of responding to stimuli in secondary cultures.

In summary, these studies demonstrate that in vitro exposure of monocytes to sublethal doses of UVB radiation modulates the various premitotic events in T-cell activation differentially and that T lymphocytes become partially activated when incubated with UVB-irradiated accessory cells. IL-2 receptor expression is relatively resistant to UVB, whereas IL-2 production appears to be the most sensitive step in the process.

Table V. Response in Secondary Culture of T Lymphocytes Stimulated with UVB-Irradiated Monocytes and Anti-CD3 Antibodies

Cells in Primary Culture[a]	Restimulated	Tritiated Thymidine Incorporation (cpm + SD)
T cells	MN + anti-CD3	12,460 ± 4271
MN + T cells + anti-CD3		2242 ± 2564
MN + T cells + anti-CD3	anti-CD3	3394 ± 281
MN + T cells + anti-CD3	MN + anti-CD3	18,542 ± 882
MN(UV) + T cells + anti-CD3	anti-CD3	375 ± 103
MN(UV) + T cells + anti-CD3	MN + anti-CD3	18,311 ± 7441

[a] One hundred thousand T cells were placed in primary culture with 2×10^4 unirradiated or UVB-irradiated (100 J/m²) MN and OKT3 for 24 h in 96-well microtiter plates. The cultures were then harvested and used as responder cells in secondary culture with MN and OKT3. T-cell proliferation was assessed after 72 h as described in *Materials and Methods*.

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